Health monitoring component of South Delta Salmon Study - Spring 1996 Conducted by the California-Nevada Fish Health Center (Ca-Nv FHC). Prepared by Kimberly True, Fish Health Biologist

In summary, both the MRFF and FRH stocks appeared normal and similar in organosomatic indices and in health profiles. Hematological parameters and histological examination did not detect distinct differences, and both groups appeared to be undergoing smoltification.

INTRODUCTION

Problem statement:

Declining chinook populations in the San Joaquin River basin has prompted a number of restoration efforts for this valuable resource and a key element of the Basin's aquatic biodiversity. Hatchery supplementation, habitat restoration, Delta operation patterns, and harvest management are some of the major parts of this restoration effort. The South Delta study is designed to evaluate the benefits of barrier operation in the head of Old River. The health and performance of experimental fish from Feather River Hatchery (FRH) and Merced River Fish Facility (MRFF) will have major effect on the quality of data generated from a mark and recapture study.

A coordinated and sustained juvenile monitoring program is essential for restoration managers as it provides timely data on survival, migration timing, and numbers of hatchery and natural smolts. In comparison, reliance on adult return and ocean harvest data as the sole assessment of restoration progress is unwise as it overlooks a critical lifestage of these animals and requires 2-4 years per broodyear to acquire. Data on the health and performance of migrating hatchery and natural smolts is an important component of a juvenile monitoring program and helps to answer questions about survival and fitness.

Background:

In addition to our basic mission of providing technical support to federal hatcheries, the USFWS California-Nevada Fish Health Center has been an active partner in health and physiology monitoring of chinook smolts in the Klamath basin since 1991. During this period we have examined the immune status, energy reserves, smolt development, and pathogen load of both natural and hatchery chinook smolts.

In discussions with CDFG Pathologist Mr.John Modin, bacterial kidney disease (BKD) was identified as a probable health concern for chinook juveniles at Feather River Hatchery (FRH). Proliferative kidney disease (PKD) is also a health concern at the Merced River Fish Facility (MRFF). Statistically-based inspections are not performed on these fish directly prior to release unless there are obvious health problems. The stress of transport, introduction into a new environment, temperature tolerance, and normal smolt development result in rapid energy depletion and a reduction in immune defense in chinook smolts (Barton et al., 1985; Sheridan, 1989).

Study Objectives:

- a. Determine incidence of infection for internal parasites (e.g. Ceratomyxa shasta, PKX agent of Proliferative Kidney Disease,) and for the bacterium Renibacterium salmoninarum in fish from FRH and MRFF upon their release from Dos Reis on May 1, 1996.
- b. Perform organosomatic analysis of sampled fish to assess general health and morphological data.
- c. Collect gill tissue for ATPase assay and plasma for triglyceride levels and stress glucose response.
- d. Perform a 24-hour saltwater challenge (30 ppm) with 12 test fish from each release group to assess osmoregulatory ability.
- e. Perform the above health and physiological monitoring (excluding SW challenge) on adipose-clipped chinook juveniles recovered from Chipps Island trawl on May $6^{\rm th}$, 5 days post release, to assess changes in health status of emigrating smolts.

METHODS

Pathogens-Kidney tissue was removed and frozen for quantification of Renibacterium salmoninarum antigen levels by Enzyme Linked Immunosorbent Assay (ELISA). Various tissues were fixed and examined histologically for parasites and tissue abnormalities. If clinical signs were present, additional samples for suspect pathogens were collected and analyzed.

Physiology- Organosomatic analysis, including leukocrit and hematocrit measurements, were performed on both groups. The organosomatic index is a method for ordered observation and reporting of the gross morphology of selected organs, hematological parameters, and size criteria of each individual. A numeric "severity" score of 0 - 3 is assigned to each tissue representing normal to severe abnormality, allowing empirical comparisons of stocks based on target tissue health at time of exam. All quantitative data are calculated to determine mean, standard deviation, standard error of mean, as well as minimum, maximum and median values. The condition factor (weight/(length)³) describes the relationship of weight to length and allows comparison of individual fish and/or populations at various body mass compositions.

Gill tissue for Adenosine Triphosphatase (ATPase) activity was collected and frozen for analysis. The ATPase assay (McCormick et al., 1989) is used to quantify enzyme activity and assess osmoregulatory function of individual fish. Plasma triglyceride and glucose samples were collected by bleeding fish, centrifuging the blood sample for plasma separation, and freezing the plasma for later analysis using the Kodak DT-60 Clinical Chemistry Analyzer.

Osmoregulation— Survival in salt-water and plasma sodium regulation was assessed by 4-hour challenge in 30 ppt artificial sea salt (900 gm Instant ocean added to 30 L of freshwater, checked by saline refractometer). Modification of the standard 24-hour challenge (Blackburn and Clarke, 1987) was necessary due to security concerns for air supply equipment at the Dos Reis site. However, the decreased challenge period of 4 hours may not be an adequate time period to assess the osmoregulatory capability of these fish.

Buckets were held in ambient water, shaded from direct sunlight, and supplied with bubbled air from a compressed air cylinder. Test fish were sub-sampled from the release group as fish were pumped from transport trucks. Fish were quickly placed in the 30 ppt salt water buckets (10 fish/bucket). A lid was securely fastened, temperature and dissolved oxygen at initialization and termination of challenge were recorded.

After 4 hrs, the surviving fish are quickly captured, euthanized, weighed, measured for total and fork length, and bled from the caudal peduncle into heparinized tubes. The tubes are centrifuged, and the plasma samples were frozen for later sodium analysis.

RESULTS

Fish from MRFF were transported to Dos Reis in two trucks, water temperature in the tanks of the vehicles was 59 and $55^{\circ}F$, and dissolved oxygen recorded at 9.0 mg/L. Feather River Hatchery (FRH) fish were transported in one truck, water temperature of $52^{\circ}F$ was recorded, and dissolved oxygen was not measured in this vehicle. River temperature was recorded at $63^{\circ}F$ at time of release.

Organosomatic Index:

Merced River Fish Facility - Thirty-three fish were collected as fish were pumped from the transport truck. No clinical signs of parasite infection or disease were present, and fish appeared healthy. Fish were reported by transport personnel to be approximately 72 to the pound. Mean weight was 5.89 grams and ranged from 3.10 - 8.40 grams. Mean Fork length was 83.76 mm and ranged from 71-95 mm. Condition factor mean was 0.83, ranged from .73-.90. External exam indicated normal values for eyes, skin, gills and fins. Internal examination of organs did not indicate abnormalities in liver, spleen, kidney or intestinal tract.

Visceral fat scores for this stock indicated 74% of the fish had fat scores of 1, indicating disposition of fatty tissue on the pyloric cecum and the upper intestine. Fat scores of zero were noted in 26% of the fish examined, indicating poor energy reserves. Hematocrit and Leukocrit values were normal at mean values of 43.4 and 0.9, respectively. Total protein, glucose and triglyceride levels were within normal ranges as well.

Feather River Hatchery - fish were held in a live box for approximately 1.5 hours before fish health examination was performed. Thirty-four fish were examined and appeared healthy, and without clinical signs of parasites and/or disease indicators. Fish were reported to be approximately 70 to the pound. FRH fish were slightly smaller than the MRFF stock with a mean weight of 5.14 grams and a range of 2.80 - 7.30 grams. Mean Fork length was 82.18 mm and ranged from 70-95 mm. Condition factor ranged from .64-.78 with a mean of 0.72 indicating this stock was leaner in body weight to length proportions than the MRFF group.

External exam indicated normal values for eyes, skin, gills and fins. Internal examination of organs did not indicate abnormalities in liver, spleen, kidney or intestinal tract. Visceral fat scores for this stock indicated 70% of the fish had fat scores of 1, indicating disposition of fatty tissue on the pyloric cecum and the upper intestine. Fat scores of zero were noted in 30% of the fish examined, indicating poor energy reserves. Food was present in the gut of 17.4% of the fish examined, indicating fish had eaten within 24-hours.

In summary, this stock was slightly smaller, leaner in body composition and had a larger percentage of fish with no obvious visceral fat. Hematocrit and Leukocrit values were within normal ranges and not significantly different that MRFF stock with a mean value of 40.30 and 1.02, respectively. Total protein, glucose and triglyceride levels were within normal ranges.

Osmoregulation:

Merced River Fish Facility - Gill ATPase values ranged from 0.69 - 4.17 mmol ADP/gm protein/hour (mean of 2.01) indicating a fairly broad range in enzyme activity among individual fish in this stock.

All fish survived the 4-hour salt water challenge, mean plasma sodium of 168.30 (ranges were 160-174 mmol Na^{\dagger}/L) indicate these fish were able to osmoregulate in 30ppt salt-water for a challenge period of 4 hours (Blackburn and Clarke, 1989). If fish were incapable of osmoregulating, even at this shortened challenge period, we would expect elevated sodium levels in as short a time as two hours.

Feather River Hatchery - Gill ATPase values for FRH stock were somewhat lower for the group and ranged from 1.15 - 2.70 mmol ADP/gm protein/hour with less variability in range of values than the MRFF, but a lower mean of 1.33 overall.

All eleven FRH fish survived the 4-hour salt water challenge, mean plasma sodium of 164.80 (range of 154-178 mmol $\mathrm{Na}^{\scriptscriptstyle +}/\mathrm{L}$) were similar to values obtained from MRFF fish and indicate osmoregulatory capability for this stock as well. Ending salinity in the FRH bucket was recorded at 20ppt, it is unknown if this was operator error or a problem with the salinity refractometer.

Percent Survival in a 4 hr challenge of 30 ppt Salt water (number of fish tested); and Plasma Sodium values (mmoles/L), reported as mean (stdev).

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	96-52-MRFF	96-51-FRH
		00.50
Total Length	88.82	88.53
K-Factor (organo fish)	.83	.72
K-Factor (SW challenged)	. 85	. 75
Ending TEMP (C)	20.5	20.5
DO (mg/L)	6.9	6.8
SALINITY (ppt)	30.0	20.0
SW Survival (%)	100	100
Plasma Sodium (mmoles/L)	168.3 (4.0)	164.8 (6.9)

It should be noted that 4-hours is a modification of the 24-hour protocol for this assay, and a decreased challenge period of 4 hours may not be adequate to assess the osmoregulatory capability of these fish by SW survival and plasma sodium levels.

Histology:

Histological examination was performed on several individual fish from both stocks to determine presence of internal parasites and associated pathological effects. In addition, pooled intestinal tissues were examined for the parasite *Ceratomyxa shasta*, which can have debilitating effects on naïve fish during downstream migration. *Ceratomyxa shasta* is endemic to the Feather River (Hendrickson et al., 1986).

Merced River Fish Facility - Various organs were examined from up to twelve MRFF fish. Early stages of the myxosporean parasite, PKX were seen in 5 of 8 kidney sections. Interstitial hyperplasia, an inflammatory response of surrounding tissue to the PKX organism, was also noted in 3/5 kidney sections. While the infections were in the early stage of development, this is a progressive disease and can cause mortality in a 4-6 week period at temperatures above 15°C (Hedrick and Kent, 1986).

No other internal parasites or pathology was noted in this group, *Ceratomyxa shasta* was not detected in the pooled intestines.

Feather River Hatchery - Up to twelve FRH fish were examined and no parasites or lesions were seen in this group. Intestine sections were negative for Ceratomyxa shasta.

Table 1.

Histological findings for internal parasites and associated pathology:

Tissue examined	96-52 MRFF	96-51 FRH
KIDNEY - PKX Agent	5/8	0/8
POOLED INTESTINE - Ceratomyxa shasta	0/16	0/16

Bacterial Kidney Disease - Renibacterium salmoninarum:

Due to sample size constraints, kidney tissue from both stocks were two-pooled and analyzed for *Renibacterium salmoninarum* antigen by ELISA using the method of Pascho and Mulcahy (1987). Results are unremarkable, with histograms for both groups indicating very low levels of antigen for this bacterial pathogen (see Figure 1 - *Renibacterium salmoninarum* ANTIGEN LEVELS FOR MERCED AND FEATHER RIVER STOCK).

Serum electrophoresis:

Serological profiles for both groups were prepared by serum electrophoresis. The profiles were similar between groups with the exception of a statistically significant difference in the F4 band, a fraction that is likely to be a beta-protein (i.e. transferrin, complement III, or fibrinogen). The biological significance of this difference in profiles is unknown.

DISCUSSION

Overall, both stocks appeared healthy; no clinical disease signs were present, organosomatic indices were comparable between groups, and hematological values were within expected ranges. The most apparent differences between groups were higher condition factors, slightly higher hematocrit values, and more visceral fat in the Merced River stock.

The FRH fish had higher Glucose and Triglyceride values, despite their lower condition factors and lower fat scores. The glucose response for this group indicates sufficient levels for stress response, and all values are within expected ranges and probably not biologically significant between groups.

Both MRFF and FRH stocks appeared to be able to osmoregulate based on Gill ATPase ranges and survival in 30ppt salt water for 4 hours while maintaining normal plasma sodium levels.

Histological examination did not reveal health problems which likely affect migration of these stocks through the Delta. While the PKX agent, a myxosporean parasite, was detected in the MRFF the early infection stage and infection levels would not have been immediately detrimental to this stock. It should be noted that this pathogen's development in the host is progressive and can cause disease and mortality with 4--6 weeks at temperatures above 15°C , therefore this pathogen could be a factor in adult survival of this stock.

Antigen for Renibacterium salmnoninarum is at low levels in both groups, indicating little risk to smolts from this bacterial pathogen. Bacterial Kidney disease (BKD) is known to be exacerbated during salt-water entry, with onset occurring in 4-8 weeks, even in stocks showing little indication for the disease during fresh water rearing (Sanders and Fryer, 1978). Without additional samples from Chipps Island trawl or during salt water residence, it is unknown whether this pathogen could be a problem for either stock, however based on this data, the risk appears low.

It should be noted that FRH fish underwent a significant temperature shock of

 $11^{\circ}(\mathrm{F})$ upon release, and that this added physiological stress could have had negative impacts on survival.

Inability to recapture these CWT fish in the Chipps Island trawl makes it difficult to draw conclusions regarding changes in health status during migration through the Delta. The fish health data presented describes a limited perspective of health status at one point in time and should not be misconstrued as a comprehensive profile of the health of these two stocks throughout their life history.

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Figures and Tables:

ELISA Histogram (Figure 1)
Organosomatic analysis data (Appendix 1)
Gill Na-K⁺-ATPase data sheets (Appendix 2)

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FIGURE 1

ELISA HISTOGRAMS

APPENDIX 1

ORGANOSOMATIC ANALYSIS

APPENDIX II

 ${\tt GILL} \ \ {\tt Na-K^+-ADENOOSINETRIPHOSPHATASE} \ \ {\tt ASSAY} \ \ {\tt DATA}$